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(21) International Application Number: PCT/US93/06464 (22) International Filing Date: 9 July 1993 (09.07.93) (30) Priority data: 07/912,294 10 July 1992 (10.07.92) US (71) Applicant: THE UNITED STATES OF AMERICA, as represented by THE SECRETARY OF THE DEPARTMENT OF HEALTH AND HUMAN SERVICES [US/US]; Box OTT, Bethesda, MD 20892 (US). (72) Inventors: KASLOW, David, C. ; 4405 Woodfield Road, Kensington, MD 20895 (US). DUFFY, Patrick, E. ; 8315 N. Brook Lane, No. 801, Bethesda, MD 20814 (US).		(74) Agents: BASTIAN, Kevin, L. et al.; Townsend and Townsend Khourie and Crew, Steuart Street Tower, 20th Floor, One Market Plaza, San Francisco, CA 94105 (US). (81) Designated States: AU, CA, JP, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report.</i>
(54) Title: TARGET ANTIGENS OF TRANSMISSION BLOCKING ANTIBODIES FOR MALARIA PARASITES (57) Abstract The present invention relates to novel methods and compositions for blocking transmission of <i>Plasmodium spp.</i> which cause malaria. In particular, P28 proteins are disclosed which, when administered to a susceptible organism, induce an immune response against a 28 kD protein on the surface of <i>Plasmodium</i> ookinetes and block transmission of malaria.		

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TARGET ANTIGENS OF TRANSMISSION BLOCKING ANTIBODIES
FOR MALARIA PARASITES

BACKGROUND OF THE INVENTION

Malaria continues to exact a heavy toll from mankind. Between 200 million to 400 million people are infected by *Plasmodium falciparum*, the deadliest of the malarial protozoans, each year. One to four million of these people die. Approximately 25 percent of all deaths of children in rural Africa between the ages of one and four years are caused by malaria.

The life cycle of the malaria parasite is complex. Infection in man begins when young malarial parasites or "sporozoites" are injected into the bloodstream of a human by a mosquito. After injection the parasite localizes in liver cells. Approximately one week after injection, the parasites or "merozoites" are released into the bloodstream to begin the "erythrocytic" phase. Each parasite enters a red blood cell in order to grow and develop. When the merozoite matures in the red blood cell, it is known as a trophozoite and, when fully developed, as a schizont. A schizont is the stage when nuclear division occurs to form individual merozoites which are released to invade other red cells. After several schizogonic cycles, some parasites, instead of becoming schizonts through asexual reproduction, develop into large uninucleate parasites. These parasites undergo sexual development.

Sexual development of the malaria parasites involves the female or "macrogametocyte" and the male parasite or "microgametocyte." These gametocytes do not undergo any further development in man. Upon ingestion of the gametocytes into the mosquito, the complicated sexual cycle begins in the midgut of the mosquito. The red blood cells disintegrate in the midgut of the mosquito after 10 to 20 minutes. The microgametocyte continues to develop through exflagellation and releases 8 highly flagellated microgametes. Fertilization

occurs with the fusion of the microgamete and a macrogamete. The fertilized parasite, which is known as a zygote, then develops into an "ookinete." The ookinete penetrates the midgut wall of the mosquito and develops into an oocyst, within which many small sporozoites form. When the oocyst ruptures, the sporozoites migrate to the salivary gland of the mosquito via the hemolymph. Once in the saliva of the mosquito, the parasite can be injected into a host, repeating the life cycle.

Malaria vaccines are needed against different stages in the parasite's life cycle, including the sporozoite, asexual erythrocyte, and sexual stages. Each vaccine against a particular life cycle stage increases the opportunity to control malaria in the many diverse settings in which the disease occurs. For example, sporozoite vaccines would fight infection immediately after injection of the parasite into the host by the mosquito. First generation vaccines of this type have been tested in humans. Asexual erythrocytic stage vaccines would be useful in reducing the severity of the disease. Multiple candidate antigens for this stage have been cloned and tested in animals and in humans.

However, as drug-resistant parasite strains render chemoprophylaxis increasingly ineffective, a great need exists for a transmission-blocking vaccine. Such a vaccine would block the portion of the parasite's life cycle that takes place in the mosquito or other arthropod vector, thus preventing even the initial infection of humans. Several surface antigens serially appear on the parasite as it develops from gametocyte to gamete to zygote to ookinete within the arthropod midgut (Rener et al., *J. Exp. Med.* 158: 976-981, 1983; Vermeulen et al., *J. Exp. Med.* 162: 1460-1476, 1985). Several of these antigens induce transmission-blocking antibodies, but each antigen has demonstrated shortcomings: either a failure to generate an immune response in a broad segment of the vaccinated population (Good et al., *Science* 242:574-577, 1988; Graves et al., *Parasite Immunol.* 10: 209-218, 1988; Graves et al., *Infect. Immun.* 56:2818-2821, 1988; Carter et al., *J. Exp. Med.* 169:135-147, 1989). For

example, monoclonal antibodies against a *P. falciparum* 25 kD a sexual stage surface protein, Pfs25, which is expressed on zygotes and ookinetes, partially block transmission of the parasite (Vermeulen et al., supra). However, partial blocking is not sufficient to arrest the spread of malaria.

The present invention fills the need for a means to completely block transmission of malaria parasites. The vaccine of the invention meets the requirements for a vaccine for controlling endemic malaria in developing countries: it induces high, long-lasting antibody titers, and can be produced in large amounts, at the lowest possible cost.

SUMMARY OF THE INVENTION

The present invention relates to methods for preventing transmission of malaria. In particular, the invention relates to methods for eliciting an immune response against parasites responsible for the disease. These methods comprise administering to a susceptible organism a pharmaceutical composition comprising a P28 protein in an amount sufficient to induce a transmission-blocking immune response.

The invention also relates to methods of preventing transmission of malaria comprising administering to a susceptible organism a pharmaceutical composition comprising a recombinant virus encoding a P28 protein in an amount sufficient to block transmission of the disease.

The invention further relates to pharmaceutical compositions comprising a pharmaceutically acceptable carrier and the P28 proteins described above.

The invention also relates to isolated nucleic acids comprising a nucleotide sequence encoding P28 proteins. These nucleic acids may be isolated from, for instance, *P. gallinaceum* or *P. falciparum*. The sequences are typically contained in an expression vector for recombinant expression of the proteins. The sequences can also be incorporated into recombinant viruses for use as vaccines or for recombinant expression of the proteins. Cell lines containing a nucleic

acid encoding the immunogenic polypeptides in an expression vector are also disclosed.

DEFINITIONS

5 The term "P28" refers to 28kD proteins expressed on the surface *Plasmodium* ookinetes. Examples of such proteins include Pgs28 and Pfs28 from *P. gallinaceum* and *P. falciparum*, respectively. The term encompasses native proteins as well as recombinantly produced modified proteins that induce a transmission blocking immune response. It also includes
10 immunologically active fragments of these proteins.

A "susceptible organism" is a *Plasmodium* host that is susceptible to malaria, for example, humans and chickens. The particular susceptible organism or host will depend upon the *Plasmodium* species.

15 The phrases "biologically pure" or "isolated" refer to material which is substantially or essentially free from components which normally accompany it as found in its native state. Thus, the isolated P28 proteins of this invention do not contain materials normally associated with their *in situ*
20 environment. Even where a protein has been isolated to a homogenous or dominant band, there are trace contaminants in the range of 5-10% of native protein which co-purify with the desired protein. Biologically pure material does not contain such endogenous co-purified protein.

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BRIEF DESCRIPTION OF THE FIGURES

Figure 1. Nonreduced (lane 1) or reduced (lane 2) immunoaffinity purified Pgs28 was size-fractionated by a 10% SDS-polyacrylamide gel. MAb IID2-B3B3 recognizes the dominant
30 band at ~34 kD (lane 1) by Western blot, but fails to recognize a band in the reduced material (Western blot data not shown).

35 Figure 2. The deduced amino acid sequence of Pgs28, compared with the published sequences of Pgs25 and Pfs25 (Kaslow, *Mol. Biochem. Parasitol.* 33:283-288, 1989). Areas of homology between the three proteins are enclosed in boxes.

Figure 3. Southern blot analysis of genomic DNA obtained from *P. falciparum* (strain 3D7) asexual stage parasites. 5 μ g DNA was digested with restriction endonuclease *Dra*I (lane 1) or *Sca*I (lane 2). The electroblotted filter was probed with the full-length open reading frame encoding Pgs28.

DESCRIPTION OF THE PREFERRED EMBODIMENT

The present invention relates to novel compositions and methods for blocking transmission of parasites responsible for malaria. The invention provides agents capable of inhibiting the life cycle of the disease-causing parasite in the mosquito midgut. The agents include P28 proteins that are useful for inducing antibodies that block transmission of the parasite, genes encoding such polypeptides, antibodies against these polypeptides, and compositions that are useful as vaccines against malaria.

The compositions of the invention can be used to block transmission of a number of parasites associated with malaria. Examples of parasites whose transmission may be blocked include the causative agents for malaria. Four species of the genus *Plasmodium* infect humans, *P. vivax*, *P. ovale*, *P. malariae*, and *P. falciparum*. In addition other *Plasmodium* species infect other animals. For instance, *P. gallinaceum* is responsible for avian malaria.

P28 Proteins

The present invention includes immunogenic polypeptides such as P28 proteins and fragments derived from the proteins that are useful for inducing an immune response when the proteins are injected into a human or other host animal. The antibodies that arise from the immune response block transmission of the parasite by interfering with the portion of the parasite's life cycle that occurs in the mosquito. For example, purified polypeptides having an amino acid sequence substantially identical to a subsequence of Pgs28 or Pfs28 may be used. Pgs28 is a *P. gallinaceum* surface protein of M_r 28,000 kD (under reducing conditions) which is immunoprecipitated from an extract of zygotes/ookinetes by

monoclonal antibodies that suppress but do not block malaria transmission (Grotendorst et al., *Infect. Immun.* 45:775-777, 1984). Pfs28 is a homolog of Pgs28 from *P. falciparum*.

5 Pgs28 is similar in structure to both Pgs25 and Pfs25: all three proteins comprise a putative secretory signal sequence, followed by four EGF-like domains and a terminal hydrophobic transmembrane region without a cytoplasmic tail. Although the three proteins share the six-cysteine motif of the EGF-like domains, the functions of these proteins may be
10 very different. EGF-like domains have been recognized in a range of proteins that have diverse functions (Davis, *New Biol.* 2:410-419, 1990).

Although Pgs28 and Pgs25 are structurally similar, they can be differentiated by their apparent M_r on SDS-PAGE
15 (28 kD for Pgs28, 25 kD for Pgs25), as well as their specific recognition by monoclonal antibodies (Grotendorst et al., *supra.*). For example, Pgs 28 is recognized by the monoclonal antibody IID2B3B3, while Pgs25 is not. Similarly, the monoclonal antibody IID2-C5I recognizes Pgs25 but not Pgs28.

20 Included among the polypeptides of the present invention are proteins that are homologs of Pgs28 and Pfs28. Such homologs, also referred to as Pgs28 polypeptides or Pfs28 polypeptides, include variants of the native proteins constructed by *in vitro* techniques, and P28 proteins from
25 parasites related to *P. gallinaceum* or *P. falciparum* that are homologous in features such as structure and relative time of expression in the parasite life cycle. One skilled in the art will appreciate, however, that for certain uses it would be advantageous to produce a Pgs28 or Pfs28 polypeptide that is
30 lacking one of the structural characteristics; for example, one may remove the transmembrane domain to obtain a polypeptide that is more soluble in aqueous solution.

The P28 proteins of the invention may be purified from parasites isolated from infected host organisms. Methods
35 for purifying desired proteins are well known in the art and are not presented in detail here. For a review of standard techniques see, *Methods in Enzymology*, "Guide to Protein Purification", M. Deutscher, ed. Vol. 182 (1990), which is

incorporated herein by reference. For instance, Pfs28, Pgs28 or their homologous polypeptides can be purified using affinity chromatography, SDS-PAGE, and the like. For example, see Example 1 for a procedure for purifying Pgs28.

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Nucleic Acids

Another aspect of the present invention relates to the cloning and recombinant expression of P28 proteins such as Pfs28 and Pgs28 obtained from the parasites discussed above. The recombinantly expressed proteins can be used in a number of ways. For instance, they can be used as transmission-blocking vaccines or to raise antibodies, as described below. In addition, oligonucleotides from the cloned genes can be used as probes to identify homologous polypeptides in other species.

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Thus, the invention relies on routine techniques in the field of recombinant genetics, well known to those of ordinary skill in the art. A basic text disclosing the general methods of use in this invention is Sambrook et al., *Molecular Cloning, A Laboratory Manual*, Cold Spring Harbor Publish., Cold Spring Harbor, NY 2nd ed. (1989).

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The steps required to clone genes encoding P28 proteins and express polypeptides suitable for the invention are well known to one of skill in the art. In summary, the manipulations necessary to prepare nucleic acid segments encoding the polypeptides and introduce them into appropriate host cells involve 1) purifying the polypeptide from the appropriate sources, 2) preparing degenerate oligonucleotide probes corresponding to a portion of the amino acid sequence of the purified proteins, 3) screening a cDNA or genomic library for the sequences which hybridize to the probes, 4) constructing vectors comprising the sequences linked to a promoter and other sequences necessary for expression and 5) inserting the vectors into suitable host cells or viruses.

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After isolation of the desired protein as described

above, the amino acid sequence of the N-terminus is determined and degenerate oligonucleotide probes, designed to hybridize to the desired gene, are synthesized. Amino acid sequencing is performed and oligonucleotide probes are synthesized according to standard techniques as described, for instance, in Sambrook et al., supra.

Oligonucleotide probes useful for identification of desired genes can also be prepared from conserved regions of related genes in other species. For instance, probes derived from a gene encoding Pgs28 from *P. gallinaceum* or Pfs28 from *P. falciparum* may be used to screen libraries for homologous genes from other parasites of interest.

Genomic or cDNA libraries are prepared according to standard techniques as described, for instance, in Sambrook, supra. To construct genomic libraries, large segments of genomic DNA are generated by random fragmentation and are ligated with vector DNA to form concatemers that can be packaged into the appropriate vector. Two kinds of vectors are commonly used for this purpose, bacteriophage lambda vectors and plasmids.

To prepare cDNA, mRNA from the parasite of interest is first isolated. Eukaryotic mRNA has at its 3' end a string of adenine nucleotide residues known as the poly-A tail. Short chains of oligo d-T nucleotides are then hybridized with the poly-A tails and serve as a primer for the enzyme, reverse transcriptase. This enzyme uses RNA as a template to synthesize a complementary DNA (cDNA) strand. A second DNA strand is then synthesized using the first cDNA strand as a template. Linkers are added to the double-stranded cDNA for insertion into a plasmid or phage vector for propagation in *E. coli*.

Identification of clones in either genomic or cDNA libraries harboring the desired nucleic acid segments is performed by either nucleic acid hybridization or immunological detection of the encoded protein, if an expression vector is used. The bacterial colonies are then replica plated on solid support, such as nitrocellulose filters. The cells are lysed and probed with either

oligonucleotide probes described above or with antibodies to the desired protein. For example, see Example 3 below, which describes the cloning of Pgs28, and Example 4, which describes cloning of Pfs28.

5 Other methods well known to those skilled in the art can also be used to identify desired genes. For example, the presence of restriction fragment length polymorphisms (RFLP) between wild type and mutant strains lacking a Pgs28 or Pfs28 polypeptide can be used. Amplification techniques, such as
10 the polymerase chain reaction (PCR) can be used to amplify the desired nucleotide sequence. U.S. Patents Nos. 4,683,195 and 4,683,202 describe this method. Sequences amplified by PCR can be purified from agarose gels and cloned into an appropriate vector according to standard techniques.

15 Standard transfection methods are used to produce prokaryotic, mammalian, yeast or insect cell lines which express large quantities of the Pgs28 or Pfs28 polypeptide, which is then purified using standard techniques. See, e.g., Colley et al., *J. Biol. Chem.* 264:17619-17622, 1989; and Guide
20 to Protein Purification, *supra*.

The nucleotide sequences used to transfect the host cells can be modified according to standard techniques to yield Pfs 28 or Pgs28 polypeptides or fragments thereof, with a variety of desired properties. The polypeptides of the
25 present invention can be readily designed and manufactured utilizing various recombinant DNA techniques well known to those skilled in the art. For example, the polypeptides can vary from the naturally-occurring sequence at the primary structure level by amino acid, insertions, substitutions,
30 deletions, and the like. These modifications can be used in a number of combinations to produce the final modified protein chain.

The amino acid sequence variants can be prepared with various objectives in mind, including facilitating
35 purification and preparation of the recombinant polypeptide. The modified polypeptides are also useful for modifying plasma half life, improving therapeutic efficacy, and lessening the severity or occurrence of side effects during therapeutic use.

The amino acid sequence variants are usually predetermined variants not found in nature but exhibit the same immunogenic activity as naturally occurring Pgs28, Pfs28, or other P28 proteins. For instance, polypeptide fragments comprising only
5 a portion (usually at least about 60-80%, typically 90-95%) of the primary structure may be produced. For use as vaccines, polypeptide fragments are typically preferred so long as at least one epitope capable of eliciting transmission blocking antibodies remains.

10 In general, modifications of the sequences encoding the homologous polypeptides may be readily accomplished by a variety of well-known techniques, such as site-directed mutagenesis (see, Gillman and Smith, *Gene* 8:81-97, 1979) and
15 Roberts, S. et al., *Nature* 328:731-734, 1987). One of ordinary skill will appreciate that the effect of many mutations is difficult to predict. Thus, most modifications are evaluated by routine screening in a suitable assay for the desired characteristic. For instance, the effect of various
20 modifications on the ability of the polypeptide to elicit transmission blocking can be easily determined using the mosquito feeding assays, described below. In addition, changes in the immunological character of the polypeptide can be detected by an appropriate competitive binding assay. Modifications of other properties such as redox or thermal
25 stability, hydrophobicity, susceptibility to proteolysis, or the tendency to aggregate are all assayed according to standard techniques.

The particular procedure used to introduce the genetic material into the host cell for expression of the
30 Pfs28 or Pgs28 polypeptide is not particularly critical. Any of the well known procedures for introducing foreign nucleotide sequences into host cells may be used. These include the use of calcium phosphate transfection, spheroplasts, electroporation, liposomes, microinjection,
35 plasma vectors, viral vectors and any of the other well known methods for introducing cloned genomic DNA, cDNA, synthetic DNA or other foreign genetic material into a host cell (see Sambrook et al., *supra*). It is only necessary that the

particular procedure utilized be capable of successfully introducing at least one gene into the host cell which is capable of expressing the gene.

5 The particular vector used to transport the genetic information into the cell is also not particularly critical. Any of the conventional vectors used for expression of recombinant proteins in prokaryotic and eukaryotic cells may be used. Expression vectors for mammalian cells typically contain regulatory elements from eukaryotic viruses. SV40
10 vectors include pSVT7 and pMT2. Vectors derived from bovine papilloma virus include pBV-1MTHA, and vectors derived from Epstein Bar virus include pHEBO, and p205. Other exemplary vectors include pMSG, pAV009/A⁺, pMT010/A⁺, pMAMneo-5, baculovirus pDSVE, and any other vector allowing expression
15 of proteins under the direction of the SV-40 early promoter, SV-40 later promoter, metallothionein promoter, murine mammary tumor virus promoter, Rous sarcoma virus promoter, polyhedrin promoter, or other promoters shown effective for expression in eukaryotic cells.

20 The expression vector typically contains a transcription unit or expression cassette that contains all the elements required for the expression of the Pgs28 or Pfs28 polypeptide DNA in the host cells. A typical expression cassette contains a promoter operably linked to the DNA
25 sequence encoding a Pgs28 or Pfs28 polypeptide and signals required for efficient polyadenylation of the transcript. The term "operably linked" as used herein refers to linkage of a promoter upstream from a DNA sequence such that the promoter mediates transcription of the DNA sequence. The promoter is
30 preferably positioned about the same distance from the heterologous transcription start site as it is from the transcription start site in its natural setting. As is known in the art, however, some variation in this distance can be accommodated without loss of promoter function.

35 The DNA sequence encoding the Pfs28 or Pgs28 polypeptide will typically be linked to a cleavable signal peptide sequence to promote secretion of the encoded protein by the transformed cell. Additional elements of the cassette

may include selectable markers, enhancers and, if genomic DNA is used as the structural gene, introns with functional splice donor and acceptor sites.

Enhancer elements can stimulate transcription up to 1,000 fold from linked homologous or heterologous promoters. Enhancers are active when placed downstream from the transcription initiation site. Many enhancer elements derived from viruses have a broad host range and are active in a variety of tissues. For example, the SV40 early gene enhancer is suitable for many cell types. Other enhancer/promoter combinations that are suitable for the present invention include those derived from polyoma virus, human or murine cytomegalovirus, the long term repeat from various retroviruses such as murine leukemia virus, murine or Rous sarcoma virus and HIV. See, *Enhancers and Eukaryotic Expression*, Cold Spring Harbor Press, Cold Spring Harbor, N.Y. 1983, which is incorporated herein by reference.

In addition to a promoter sequence, the expression cassette should also contain a transcription termination region downstream of the structural gene to provide for efficient termination. The termination region may be obtained from the same gene as the promoter sequence or may be obtained from different genes.

If the mRNA encoded by the structural gene is to be efficiently translated, polyadenylation sequences are also commonly added to the vector construct. Two distinct sequence elements are required for accurate and efficient polyadenylation: GU or U rich sequences located downstream from the polyadenylation site and a highly conserved sequence of six nucleotides, AAUAAA, located 11-30 nucleotides upstream. Termination and polyadenylation signals that are suitable for the present invention include those derived from SV40, or a partial genomic copy of a gene already resident on the expression vector.

Efficient expression and secretion in yeast is conveniently obtained using expression vectors based on those disclosed in Barr et al., *J. Biol. Chem.* 263: 16471-16478, 1988, or U.S. Patent No. 4,546,082, which are incorporated

herein by reference. In these vectors the desired sequences are linked to sequences encoding the yeast α -factor pheromone secretory signal/leader sequence. Suitable promoters to use include the ADH2/GAPDH hybrid promoter as described in Cousens et al., Gene 61:265-275 (1987), which is incorporated herein by reference. Yeast cell lines suitable for the present invention include BJ 2168 (Berkeley Yeast Stock Center) as well as other commonly available lines.

Any of a number of other well known cells and cell lines can be used to express the polypeptides of the invention. For instance, prokaryotic cells such as *E. coli* can be used. Eukaryotic cells include, Chinese hamster ovary (CHO) cells, COS cells, mouse L cells, mouse A9 cells, baby hamster kidney cells, C127 cells, PC8 cells, and insect cells.

Following the growth of the recombinant cells and expression of the Pfs28 or Pgs28 polypeptide, the culture medium is harvested for purification of the secreted protein. The media are typically clarified by centrifugation or filtration to remove cells and cell debris and the proteins are concentrated by adsorption to any suitable resin such as, for example, CDP-Sepharose, Asialoproteothrombin-Sepharose 4B, or Q Sepharose, or by use of ammonium sulfate fractionation, polyethylene glycol precipitation, or by ultrafiltration. Other routine means known in the art may be equally suitable. Further purification of the Pgs28 polypeptide can be accomplished by standard techniques, for example, affinity chromatography, ion exchange chromatography, sizing chromatography or other protein purification techniques to obtain homogeneity. The purified proteins are then used to produce pharmaceutical compositions, as described below.

Transmission-blocking Antibodies

A further aspect of the invention includes antibodies against Pgs28, Pfs28, or their homologous polypeptides. The antibodies are useful for blocking transmission of parasites. Importantly, the antibodies of the invention are polyclonal and thus are capable of blocking parasite transmission, in contrast to monoclonal antibodies to

Pgs28, which reduce but do not eliminate infectivity (Grotendorst et al., *supra.*).

Antibodies are typically tetramers of immunoglobulin polypeptides. As used herein, the term "antibody" refers to a protein consisting of one or more polypeptides substantially encoded by immunoglobulin genes. Immunoglobulin genes include those coding for the light chains, which may be of the kappa or lambda types, and those coding for the heavy chains. Heavy chain types are alpha, gamma, delta, epsilon and mu. The carboxy terminal portions of immunoglobulin heavy and light chains are constant regions, while the amino terminal portions are encoded by the myriad immunoglobulin variable region genes. The variable regions of an immunoglobulin are the portions that provide antigen recognition specificity. The immunoglobulins may exist in a variety of forms including, for example, Fv, Fab, and F(ab)₂, as well as in single chains (e.g., Huston et al., *Proc. Natl. Acad. Sci. USA*, 85:5879-5883 (1988) and Bird et al., *Science* 242: 423-426, 1988, both of which are incorporated herein by reference). (See, generally, Hood et al., *Immunology*, Benjamin, N.Y., 2nd ed. (1984), and Hunkapiller and Hood, *Nature*, 323: 15-16, 1986, which are incorporated herein by reference). Single-chain antibodies, in which genes for a heavy chain and a light chain are combined into a single coding sequence, may also be used.

Vaccines

The immunoglobulins, nucleic acids, and polypeptides of the present invention are also useful as prophylactics, or vaccines, for blocking transmission of malaria or other diseases caused by parasites. Compositions containing the immunoglobulins, polypeptides, or a cocktail thereof are administered to a subject, giving rise to an anti-Pgs28 or anti-Pfs28 polypeptide immune response in the mammal entailing the production of anti-Pgs28 or anti-Pfs28 polypeptide immunoglobulins. The Pgs28 or Pfs28 polypeptide-specific immunoglobulins then block transmission of the parasite from the subject to the arthropod vector, preventing the parasite from completing its life cycle. An amount of prophylactic

composition sufficient to result in blocking of transmission is defined to be an "immunologically effective dose."

The isolated nucleic acid sequences coding for Pgs28, Pfs28, or their homologous polypeptides can also be used to transform viruses which transfect host cells in the susceptible organism. Live attenuated viruses, such as vaccinia or adenovirus, are convenient alternatives for vaccines because they are inexpensive to produce and are easily transported and administered. Vaccinia vectors and methods useful in immunization protocols are described in, e.g., U.S. Patent No. 4,722,848, incorporated herein by reference.

Suitable viruses for use in the present invention include, but are not limited to, pox viruses, such as, canarypox and cowpox viruses, and vaccinia viruses, alpha viruses, adenoviruses, and other animal viruses. The recombinant viruses can be produced by methods well known in the art: for example, using homologous recombination or ligating two plasmids together. A recombinant canarypox or cowpox virus can be made, for example, by inserting the gene encoding the Pgs28, Pfs28, or other homologous polypeptide into a plasmid so that it is flanked with viral sequences on both sides. The gene is then inserted into the virus genome through homologous recombination.

A recombinant adenovirus virus can be produced, for example, by ligating two plasmids each containing 50% of the viral sequence and the DNA sequence encoding the Pgs28, Pfs28, or other homologous polypeptide. Recombinant RNA viruses such as the alpha virus can be made via a cDNA intermediate using methods known in the art.

The recombinant virus of the present invention can be used to induce anti-Pfs28 or anti-Pgs28 polypeptide antibodies in mammals, such as mice or humans. In addition, the recombinant virus can be used to produce the Pgs28 or Pfs28 polypeptides by infecting host cells which in turn express the polypeptide.

The present invention also relates to host cells infected with the recombinant virus of the present invention.

The host cells of the present invention are preferably eukaryotic, such as yeast cells, or mammalian, such as BSC-1 cells. Host cells infected with the recombinant virus express the Pgs28 or Pfs28 polypeptides on their cell surfaces. In addition, membrane extracts of the infected cells induce transmission blocking antibodies when used to inoculate or boost previously inoculated mammals.

In the case of vaccinia virus (for example, strain WR), the sequence encoding the Pgs28 or Pfs28 polypeptides can be inserted into the viral genome by a number of methods including homologous recombination using a transfer vector, pTKgpt-OFIS as described in Kaslow et al., *Science* 252:1310-1313, 1991, which is incorporated herein by reference.

The Pfs28 or Pgs28 polypeptides, or recombinant viruses of the present invention can be used in pharmaceutical and vaccine compositions that are useful for administration to mammals, particularly humans, to block transmission of a variety of infectious diseases. The compositions are suitable for single administrations or a series of administrations. When given as a series, inoculations subsequent to the initial administration are given to boost the immune response and are typically referred to as booster inoculations.

The pharmaceutical compositions of the invention are intended for parenteral, topical, oral or local administration. Preferably, the pharmaceutical compositions are administered parenterally, e.g., intravenously, subcutaneously, intradermally, or intramuscularly. Thus, the invention provides compositions for parenteral administration that comprise a solution of the agents described above dissolved or suspended in an acceptable carrier, preferably an aqueous carrier. A variety of aqueous carriers may be used, e.g., water, buffered water, 0.4% saline, 0.3% glycine, hyaluronic acid and the like. These compositions may be sterilized by conventional, well known sterilization techniques, or may be sterile filtered. The resulting aqueous solutions may be packaged for use as is, or lyophilized, the lyophilized preparation being combined with a sterile solution prior to administration. The compositions may contain

pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions, such as pH adjusting and buffering agents, tonicity adjusting agents, wetting agents and the like, for example, sodium acetate, sodium lactate, sodium chloride, potassium chloride, calcium chloride, sorbitan monolaurate, triethanolamine oleate, etc.

For solid compositions, conventional nontoxic solid carriers may be used which include, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin, talcum, cellulose, glucose, sucrose, magnesium carbonate, and the like. For oral administration, a pharmaceutically acceptable nontoxic composition is formed by incorporating any of the normally employed excipients, such as those carriers previously listed, and generally 10-95% of active ingredient and more preferably at a concentration of 25%-75%.

For aerosol administration, the polypeptides or recombinant viruses are preferably supplied in finely divided form along with a surfactant and propellant. The surfactant must, of course, be nontoxic, and preferably soluble in the propellant. Representative of such agents are the esters or partial esters of fatty acids containing from 6 to 22 carbon atoms, such as caproic, octanoic, lauric, palmitic, stearic, linoleic, linolenic, olesteric and oleic acids with an aliphatic polyhydric alcohol or its cyclic anhydride. Mixed esters, such as mixed or natural glycerides may be employed. A carrier can also be included, as desired, as with, e.g., lecithin for intranasal delivery.

In therapeutic applications, Pfs 28 or Pgs28 polypeptides or viruses of the invention are administered to a patient in an amount sufficient to prevent parasite development in the arthropod and thus block transmission of the disease. An amount adequate to accomplish this is defined as a "therapeutically effective dose." Amounts effective for this use will depend on, e.g., the particular polypeptide or virus, the manner of administration, the weight and general state of health of the patient, and the judgment of the prescribing physician.

The vaccines of the invention contain as an active ingredient an immunogenically effective amount of the Pgs28 or Pfs28 polypeptides or recombinant virus as described herein. Useful carriers are well known in the art, and include, e.g., thyroglobulin, albumins such as human serum albumin, tetanus toxoid, polyamino acids such as poly(D-lysine:D-glutamic acid), influenza, hepatitis B virus core protein, hepatitis B virus recombinant vaccine and the like. The vaccines can also contain a physiologically tolerable (acceptable) diluent such as water, phosphate buffered saline, or saline, and further typically include an adjuvant. Adjuvants such as incomplete Freund's adjuvant, aluminum phosphate, aluminum hydroxide, or alum are materials well known in the art.

Vaccine compositions containing the polypeptides or viruses of the invention are administered to a patient to elicit a transmission-blocking immune response against the antigen and thus prevent spread of the disease through the arthropod vector. Such an amount is defined as an "immunogenically effective dose." In this use, the precise amounts again depend on the patient's state of health and weight, the mode of administration, and the nature of the formulation.

The following examples are offered by way of illustration, not by way of limitation.

EXAMPLE 1

LOCALIZATION OF Pgs28

Methods

To label Pgs28, mature ookinetes were fixed for 30 min at 4°C in 0.1% glutaraldehyde and 4% formaldehyde in phosphate-buffered saline (PBS). Parasites were then incubated with ascites containing mAb IID2-B3B3, an IgG¹ antibody. Ascites was diluted 1:20 in PBS with 1% BSA. After 5 washes with PBS/BSA, the cells were incubated with goat anti-mouse antibodies (EY Labs, Inc., San Mateo, CA) conjugated with colloidal gold (10-15 nm), then washed 3 times with PBS.

Ookinetes were post-fixed in 1% osmium tetroxide in 0.1 M cacodylate buffer solution containing 2 mM CaCl_2 and 0.8% potassium ferricyanide, dehydrated in acetone, and embedded in Epon. Routine thin sections were stained with uranyl acetate and lead citrate, then visualized (post-fixing, embedding, and visualization by Dr. Paulo Pimenta, Laboratory of Parasitic Diseases, NIH, Bethesda, MD).

Results

Immunoelectron microscopy, using mAb IID2 B3B3, demonstrated the uniform and extensive distribution of Pgs28 in both the longitudinal and presumed transverse sections of the mature ookinete. This corroborates earlier biosynthetic work that showed that Pgs25 achieves peak synthesis in the early hours after parasite fertilization, then is exceeded about 10 hours after fertilization by expression of Pgs28, which becomes the predominant surface protein of mature ookinetes (Kumar et al., *Mol. Biochem. Parasitol.* 14:127-139, 1985).

EXAMPLE 2

PURIFICATION OF Pgs28

Methods

Ookinete antigens. Purified zygotes of *P. gallinaceum* were prepared from the parasitized blood of infected White Leghorn chickens as previously described (Kaushal et al., *J. Immunol.* 131:2557-2562, 1983). Zygotes were transformed in vitro into ookinetes by incubation ($1 \times 10^7/\text{ml}$) for 24 hr at 26°C in Medium 199 with 17 mM dextrose, 1 mM L-glutamine, 100 U/ml penicillin, and 100 $\mu\text{g}/\text{ml}$ streptomycin, pH 8.4. Morgan et al. *Proc. Soc. Exp. Biol. Med.* 73: (1950). Antigens were extracted with NETT buffer: 50 mM Tris, 150 mM NaCl, 5 mM EDTA, 0.5% Triton X-100, 0.02% NaN_3 , pH 7.4.

Pgs28 Purification. Pgs28 was immunoaffinity-purified from ookinete extracts using

monoclonal antibody IID2-B3B3 (Grotendorst et al., *supra.*) covalently linked to Sepharose 4B beads (mAb covalently attached to Protein A by bifunctional cross-linker) in a column, (protocol for column use in manufacturer's literature ImmunoPure® Kit, Pierce, Rockford IL). The resin with bound Pgs28 was suspended in electroelution buffer (50 mM NH_4HCO_3 , 0.1% SDS), and Pgs28 electroeluted from the resin for 4 h at 10 mA. The sample containing Pgs28 was concentrated in a Speed Vac® dessicator or Amicon Centricon® 10 microconcentrater, diluted 1:1 with SDS-PAGE sample buffer (8% SDS, 3.0 M Tris-HCl, pH 8.45, 24% glycerol, 0.015% Serva Blue G, and 0.005% Phenol Red), and size-fractionated by SDS-PAGE in a 10% polyacrylamide gel under nonreducing or reducing conditions. Pgs28 was electroblotted from the gel onto pure nitrocellulose, *in situ* digested with trypsin (Matsudaira, J. Biol. Chem. 262:10035-10038, 1987) and microsequenced (Bill Lane, Harvard MicroChemistry, Cambridge, MA) or electroblotted onto PVDF for N-terminal sequence (John Coligan, Biological Resources Branch, NIH, Bethesda, MD).

20

Results

Immunoaffinity purification of Pgs28 from crude ookinete extract resulted in a dominant band of M_r 34,000 on 10% SDS-PAGE (Fig. 1), which was electroblotted onto polyvinylidene difluoride for N-terminal sequencing of the mature protein. β mercaptoethanol reduction of the immunoaffinity-purified material caused Pgs28 to comigrate on SDS-PAGE with the small amount of mouse light chain that co-eluted from the immunoaffinity column. After blotting onto nitrocellulose, the protein was digested with trypsin, and eluted peptides separated by reverse phase high pressure liquid chromatography. Three tryptic peptides were sequenced, of which two (called NT14 and NT16) were unique when screened in Swiss Prot (Release 17, Centre Medicale Universitaire, Geneva, Switzerland) and one was substantially identical to the mouse antibody light chain.

35

Pgs28 and Pgs25, in addition to having different molecular weights, can also be differentiated by their

specific recognition by monoclonal antibodies (Grotendorst et al., *supra.*). By Western blot analysis (data not shown), Triton X-100 extracts of ookinetes depleted of Pgs28 by chromatography with mAb IID2-B3B3 (specific for Pgs28) were not depleted of Pgs25 as assayed by mAb IID2-C5I (specific for Pgs25). Furthermore, the immunoaffinity-purified Pgs28 (Fig. 1) was, by Western blot analysis, recognized by IID2 B3B3, but not by mAb IID2-C5I.

EXAMPLE 3

CLONING OF Pgs28 GENE

Methods

Screening genomic DNA library. The amino acid sequences of peptides from tryptic digests of Pgs28 were used to derive synthetic degenerate oligonucleotide probes, which were synthesized on an Applied Biosystems Inc. automated synthesizer. A HindIII-digested genomic library of *P. gallinaceum* DNA was constructed in pUC13 and electroporated into *E. coli*. The colonies were screened with the probe NT14AGT (5'-TT (AG)TT (AG)TC (TC)TT GTA TGG (AG)TC (TC)TC-3') by hybridizing at 45°C for 16 h and washing the filters at a final stringency of 6 x SSC (= 1 M sodium chloride, 0.1 M sodium citrate, pH 7.0), 0.1% SDS at 49°C for 5 m. Autoradiography at -70°C for 4-16 h was performed to identify positive colonies. Using the probes, as well as other synthetic oligonucleotides as sequencing primers, the nucleotide sequence for positive colonies was determined by the dideoxynucleotide terminator method.

Results

Completely degenerate oligonucleotide probes based on the Pfs28 amino acid sequences obtained in Example 2 were used to probe total RNA from *P. gallinaceum* zygotes that had grown for six hours. The probes hybridized to a 1.4 Kb transcript. However, these probes failed to detect the gene by either Southern blot hybridization with genomic digests or colony screening of existing cDNA and genomic libraries. To increase specificity, the antisense oligonucleotide based on

peptide NT14 was synthesized without degeneracy at positions 12 (either A or G used) and 15 (where each of the four nucleotides was used in separate constructs), then hybridized with a Northern blot of total RNA obtained from 6 hour old zygotes. A greatly enhanced signal occurred with guanosine at position 12 and thymidine at position 15. This probe (NT14AGT) identified a 3.3 kB band on Southern blot hybridization of a *Hind*III digest of *P. gallinaceum* genomic DNA, and subsequently identified a positive clone (clone 9A1) in a library of *Hind*III-digested genomic DNA ligated into pUC13.

Clone 9A1 was sequenced, and found to have a 666 bp open reading frame (see, Fig. 2 and SEQ ID nos. 1 and 2). All three previously sequenced peptides were included in the resulting deduced amino acid sequence, the only misread occurring at position 8 of the N terminus (sequenced as proline; deduced as cysteine). The structural homology between Pgs28 and both Pgs25 and Pfs25 is considerable; all three proteins have a putative secretory signal sequence, then four EGF-like domains, and a terminal hydrophobic transmembrane region without a cytoplasmic tail. Although the 6 cysteine motif of the EGF-like domains is shared between these proteins, this does not suggest a shared function. These domains have been recognized in a range of proteins with a diversity of functions (Davis, *New Biol.* 2:410-419, 1990).

EXAMPLE 4

CLONING OF Pfs28 GENE

Methods

The Pgs28 gene was amplified by polymerase chain reaction using primers that flank the open reading frame in clone 9A1. This fragment was radiolabelled and used to probe genomic DNA from asexual stage *P. falciparum* (strain 3D7) or *P. gallinaceum* parasites. The DNA from the parasites was electrophoresed through 1% agarose gel and transferred to nylon. Filters were hybridized overnight at $T_m - 10^\circ\text{C}$ with ^{32}P -labelled probes, then washed with 6 x SSC, 0.1% SDS at

T_m -5°C for 5 mins (Southern blots) or 7 mins (Northern blots). Autoradiographs were developed after 4-16 h exposure at -70°C.

Results

5 When hybridized with restriction endonuclease-digested genomic DNA from *P. falciparum*, the Pgs28 probe hybridized to a unique band (Fig. 3). The restriction digestion pattern was distinct from that seen with Pfs25 probes.

10 The Pgs28 probe is then used to screen a genomic or cDNA library from *P. falciparum* to clone the gene for Pfs28. Alternatively, the above-identified fragment is eluted from the gel and cloned into a cloning vector by methods well known to those skilled in the art. The Pfs28 gene is then sequenced
15 by a commonly used method, and is operably linked to a promoter in an expression vector. The Pfs28 polypeptide produced by the expression vector in an appropriate host cell is then used, for example, as a vaccine to elicit an immune response that blocks transmission of *P. falciparum*. The
20 polypeptide is also useful in the other means described herein for Pgs28 polypeptides.

EXAMPLE 5

BLOCKING OF *P. GALLINACEUM* TRANSMISSION

25 *Immunizations.* Pgs28 contained in polyacrylamide gel was dispersed in Ribi Adjuvant System (RAS) emulsion (MPL®+TDM) according to the manufacturer's protocol (Ribi ImmunoChem Research, Inc., Hamilton, MN). Male BALB/c mice, aged 4-6 weeks, were immunized intraperitoneally with 0.2 ml
30 emulsion for primary immunization, and again at 3 and 6 weeks for boosting immunization. The control group of mice received polyacrylamide gel without antigen dispersed in adjuvant.

35 *Transmission-blocking assays for malaria.* The method of quantifying transmission-blocking antibodies in vitro was generally as described in Quakyi et al., *J. Immunol.* 139:4213, 1987, which is incorporated herein by reference. Briefly, mosquitoes were fed on *P. falciparum*-parasitized

material (either infected blood or mature ookinetes mixed with naive blood) through a membrane. Infectivity was measured 1 week after feeding by counting the number of oocysts per mosquito midgut of 20 mosquitoes. By adding post-immunization mouse sera (diluted in heat-inactivated normal chicken serum) to the parasitized blood, we measured the effect of the sera on parasite transmission. If the addition of immune sera reduced infectivity compared with the control then the immune sera demonstrated transmission-blocking antibodies.

Statistical analysis. We analyzed two endpoints of transmission-blocking antibodies: the percentage of mosquitoes in a batch that had one or more oocysts on their midgut, and the number of oocysts per midgut. Mosquito batches fed on blood containing immune sera were compared with those fed on blood with control sera. The percentage of mosquitoes with oocysts was compared by Chi-square analysis. The number of oocysts/midgut was compared by Wilcoxon's rank sum analysis.

Results

Polyclonal, monospecific antisera from mice immunized with immunoaffinity-purified Pgs28 completely blocks *P. gallinaceum* transmission. Mosquitoes which received α Pgs28 antisera in addition to parasitized chicken blood developed significantly fewer oocysts compared to those mosquitoes which received either pre-immune or control sera (Table 1 A,B, and C). In fact, in three transmission-blocking assays, 47 mosquitoes received α Pgs28 antisera, of which only a single mosquito was infected, and in that mosquito only a single oocyst developed.

Table 1. Transmission-blocking activity of sera from immunized animals

	Sample	Mean oocyst number (range)	Infectivity percent of prebleed	Mosq. infected\ Mosq. dissected
5	A.			
	Pre-immune	39.2 (21-62)		4/4
10	Anti-Pgs28	0(0)	0%	0/5
	B.			
	Control	6.5 (0-30)		17/22
15	Anti-Pgs28	0.04 (0-1)	<0.01%	1/23
	C.			
	Control	13.0 (5-26)		6/6
20	Anti-Pgs28	0 (0)	0%	0/20
	D.			
	Control	33.5 (0-302)		17/20
	Anti-Pgs28	4.2 (0-56)	12.5%	7/21

Polyclonal antisera against Pgs28 impairs at least two distinct stages of parasite sexual development. During an overnight incubation in M199, *P. gallinaceum* zygotes readily transform into elongated ookinetes, reproducing the events which naturally occur in the mosquito midgut. The addition of α Pgs28 antisera significantly reduced the proportion of parasites which underwent this *in vitro* transformation (Table 2). *In vivo*, the ookinete traverses the midgut epithelium, then lodges beneath the basal lamina to develop into an oocyst. This development can be accomplished by feeding mature ookinetes (grown in M199) to mosquitoes; however, the proportion of mosquitoes which develop oocysts was significantly reduced by adding α Pgs28 antisera to *in vitro* ookinetes (Table 1D). As the incubation of mature ookinetes with α Pgs28 antisera *in vitro* did not induce parasite death (data not shown), the explanation(s) for the antibody effects remains unclear.

Table 2. *In vitro* transformation-blocking activity of sera from immunized animals

5	Sample	Number of Ookinetes	Total number of parasites	Percent Transformation
10	A.			
	Control	65	129	50.4%
	Anti-Pgs28	10	155	6.5%
	B.			
	Control	55	143	38.5%
	Anti-Pgs28	16	109	14.7%
15	C.			
	Control	36	101	35.6%
	Anti-Pgs28	5	104	<1.0%

Monoclonal antibodies to Pgs28 have previously been shown to suppress the number of oocysts that developed after an infectious bloodmeal, reducing infectivity (measured as mean number oocysts/midgut) to 38-48% of control (Grotendorst et al., supra.). The clear superiority of polyclonal antisera over the prior art monoclonal antibodies, as demonstrated herein, may represent the combined result of multiple blocks in parasite development.

The invention has been described in these examples and the above disclosure in some detail for the purposes of clarity and understanding. It will be apparent, however, that certain changes and modifications may be practiced within the scope of the appended claims.

SEQUENCE LISTING

- 5 (1) GENERAL INFORMATION:
- (i) APPLICANT: Kaslow, David C.
Duffy, Patrick E.
- 10 (ii) TITLE OF INVENTION: Target Antigens of Transmission
Blocking
Antibodies for Malaria Parasites
- (iii) NUMBER OF SEQUENCES: 2
- 15 (iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Townsend and Townsend
(B) STREET: One Market Plaza
(C) CITY: San Francisco
20 (D) STATE: California
(E) COUNTRY: USA
(F) ZIP: 94105
- (v) COMPUTER READABLE FORM:
25 (A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25
- 30 (vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: US
(B) FILING DATE:
(C) CLASSIFICATION:
- 35 (viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Bastian, Kevin L.
(B) REGISTRATION NUMBER: 34,677
(C) REFERENCE/DOCKET NUMBER: 15280-46
- 40 (ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 415-543-9600
(B) TELEFAX: 415-543-5043

28

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 858 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

10

(ix) FEATURE:

- 15 (A) NAME/KEY: CDS
(B) LOCATION: 123..788

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

20 TTTTTTGTCA TATTATTATC ATTTTAAAT TCATTCTAT TTCCCATAAT
AAATTATTCT 60

ACAAAATATT CAAACGAAGA TTATTTAGTA AACGAAAACA ATTTTAACAT
TTATTTAAAA 120

25 AA ATG AAA ATT CCT AGT TTA TAT TTT TTC TTT TTT ATT CAA ATT GCA
167
Met Lys Ile Pro Ser Leu Tyr Phe Phe Phe Phe Ile Gln Ile Ala
1 5 10 15

30 ATA ATA TTA ACT ATT GCA GCT CCT TCA GAT GAT GAA CCT TGT AAA AAT
215
Ile Ile Leu Thr Ile Ala Ala Pro Ser Asp Asp Glu Pro Cys Lys Asn
35 20 25 30

40 GGT TAT TTA ATA GAG ATG AGC AAT CAT ATT GAG TGC AAA TGT AAT AAT
263
Gly Tyr Leu Ile Glu Met Ser Asn His Ile Glu Cys Lys Cys Asn Asn
35 40 45

45 GAC TAT GTA TTA ACG AAT CGT TAT GAG TGT GAA CCA AAA AAT AAA TGT
311
Asp Tyr Val Leu Thr Asn Arg Tyr Glu Cys Glu Pro Lys Asn Lys Cys
50 50 55 60

55 ACA AGT TTA GAA GAT ACA AAT AAA CCT TGT GCT GAC TAT GCT AGA TGT
359
Thr Ser Leu Glu Asp Thr Asn Lys Pro Cys Ala Asp Tyr Ala Arg Cys

29

	65		70		75	
5	CTT GAG GAT CCA TAC AAA GAT AAT AAA AGT AAT TTT TAT TGC CTA TGT					
	407					
	Leu Glu Asp Pro Tyr Lys Asp Asn Lys Ser Asn Phe Tyr Cys Leu Cys					
	80		85		90	95
10	AAT AGA GGT TAT ATT CAA TAT GAA GAT AAA TGT ATT CAA GCG GAA TGT					
	455					
	Asn Arg Gly Tyr Ile Gln Tyr Glu Asp Lys Cys Ile Gln Ala Glu Cys					
15		100		105		110
	AAT TAT AAG GAA TGT GGA GAA GGA AAA TGT GTA TGG GAT GGA ATA CAT					
	503					
20	Asn Tyr Lys Glu Cys Gly Glu Gly Lys Cys Val Trp Asp Gly Ile His					
		115		120		125

30

5 GAG GAT GGT GCA TTT TGT TCA TGT AAT ATT GGT AAA GTC ATA AAT CCA
551
Glu Asp Gly Ala Phe Cys Ser Cys Asn Ile Gly Lys Val Ile Asn Pro
130 135 140

10 GAA GAT AAT AAT AAA TGC ACA AAA GAC GGA GAT ACT AAA TGT ACA CTA
599
Glu Asp Asn Asn Lys Cys Thr Lys Asp Gly Asp Thr Lys Cys Thr Leu
145 150 155

15 GAA TGT GCA CAA GGC AAG AAA TGC ATA AAA CAT GAT GTG TAT TAT ATG
647
Glu Cys Ala Gln Gly Lys Lys Cys Ile Lys His Asp Val Tyr Tyr Met
20 160 165 170 175

25 TGT GGT AAT GAT AAT TCT GGG TCT GGG TCT GGT GGT GGT GGT GGT GGT
695
Cys Gly Asn Asp Asn Ser Gly Ser Gly Ser Gly Gly Gly Gly Gly Gly
180 185 190

30 GGT AAC AGC CCA CCT CCT AGC AGT GGT AAT AGC ACC TTA TCC CTT TTC
743
Gly Asn Ser Pro Pro Pro Ser Ser Gly Asn Ser Thr Leu Ser Leu Phe
35 195 200 205

40 AAT GCA TTA AAT ATA GTT TTC TTA ATA GCT GTA ATT TAT ATC ATT
788
Asn Ala Leu Asn Ile Val Phe Leu Ile Ala Val Ile Tyr Ile Ile
210 215 220

TAAATATATG GCTGCACTTA ATGAAAGTAA TATAATTACC AGACCAAATT
AAATCATAAT 848

45 TATATGCACT
858

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 222 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

10

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Lys Ile Pro Ser Leu Tyr Phe Phe Phe Phe Ile Gln Ile Ala Ile
 15 1 5 10 15
 Ile Leu Thr Ile Ala Ala Pro Ser Asp Asp Glu Pro Cys Lys Asn Gly
 20 20 25 30
 Tyr Leu Ile Glu Met Ser Asn His Ile Glu Cys Lys Cys Asn Asn Asp
 25 35 40 45
 Tyr Val Leu Thr Asn Arg Tyr Glu Cys Glu Pro Lys Asn Lys Cys Thr
 30 50 55 60
 Ser Leu Glu Asp Thr Asn Lys Pro Cys Ala Asp Tyr Ala Arg Cys Leu
 35 65 70 75 80
 Glu Asp Pro Tyr Lys Asp Asn Lys Ser Asn Phe Tyr Cys Leu Cys Asn
 40 85 90 95
 Arg Gly Tyr Ile Gln Tyr Glu Asp Lys Cys Ile Gln Ala Glu Cys Asn
 45 100 105 110
 Tyr Lys Glu Cys Gly Glu Gly Lys Cys Val Trp Asp Gly Ile His Glu
 50 115 120 125
 Asp Gly Ala Phe Cys Ser Cys Asn Ile Gly Lys Val Ile Asn Pro Glu
 55 130 135 140

32

Asp Asn Asn Lys Cys Thr Lys Asp Gly Asp Thr Lys Cys Thr Leu Glu
145 150 155 160

5 Cys Ala Gln Gly Lys Lys Cys Ile Lys His Asp Val Tyr Tyr Met Cys
165 170 175

10 Gly Asn Asp Asn Ser Gly Ser Gly Ser Gly Gly Gly Gly Gly Gly Gly
180 185 190

15 Asn Ser Pro Pro Pro Ser Ser Gly Asn Ser Thr Leu Ser Leu Phe Asn
195 200 205

20 Ala Leu Asn Ile Val Phe Leu Ile Ala Val Ile Tyr Ile Ile
210 215 220

WHAT IS CLAIMED IS:

1. A method of preventing transmission of malaria comprising administering to a susceptible organism a pharmaceutical composition comprising a P28 protein in an amount sufficient to induce a transmission-blocking immune response.
2. The method of claim 1, wherein the P28 protein is Pgs28.
3. The method of claim 1, wherein the P28 protein is recombinantly produced.
4. The method of claim 1, wherein the susceptible organism is a chicken.
5. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and a P28 protein in an amount sufficient to induce a transmission blocking immune response in a susceptible organism.
6. The composition of claim 5, wherein the P28 protein is Pgs28.
7. The composition of claim 5, wherein the P28 protein is recombinantly produced.
8. A method of preventing transmission of malaria comprising administering to a susceptible organism a pharmaceutical composition comprising a recombinant virus encoding a P28 protein in an amount sufficient to induce a transmission blocking immune response.
9. The method of claim 8, wherein the P28 protein is Pgs28.
10. The method of claim 8, wherein the P28 protein is recombinantly produced.

11. The method of claim 8, wherein the susceptible organism is a chicken.

12. A pharmaceutical composition comprising a
5 pharmaceutically acceptable carrier and a recombinant virus encoding a P28 protein in an amount sufficient to induce a transmission blocking immune response in a susceptible organism.

10 13. The composition of claim 12, wherein the P28 protein is Pgs28.

14. The composition of claim 12, wherein the P28
15 protein is recombinantly produced.

15. A composition comprising an isolated nucleic acid encoding a P28 protein capable of eliciting a transmission blocking immune response in a susceptible organism.

20 16. A nucleic acid of claim 15, wherein the P28 protein is Pgs28.

17. A cell line containing a nucleic acid of claim
15.
25

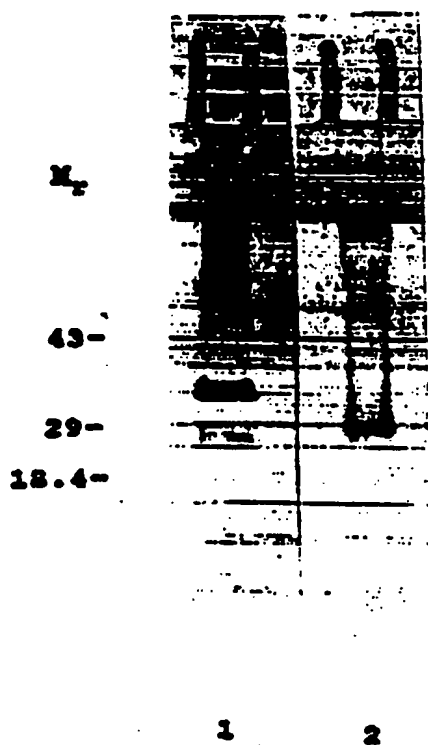


Fig. 1

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[illegible]

Figure 2

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3/3

6.5 kB

3.3 kB

.65 kB



Fig. 3

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INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 93/06464

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) ⁶		
According to International Patent Classification (IPC) or to both National Classification and IPC		
Int.Cl. 5 C12N15/11; A61K39/015; C12N15/86; A61K48/00 C12N5/10		
II. FIELDS SEARCHED		
Minimum Documentation Searched ⁷		
Classification System	Classification Symbols	
Int.Cl. 5	C12N ; A61K	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ⁸		
III. DOCUMENTS CONSIDERED TO BE RELEVANT⁹		
Category ¹⁰	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
Y	MOLECULAR AND BIOCHEMICAL PARASITOLOGY vol. 17, no. 3, December 1985, AMSTERDAM, THE NETHERLANDS pages 343 - 358 N. KUMAR 'Phase separation in Triton X-114 of antigens of transmission-blocking immunity in Plasmodium gallinaceum.' cited in the application see abstract; figure 1 see page 344, line 14 - line 17 see page 357, line 1 - line 17 ---	1-17
Y	WO,A,8 910 936 (THE UNITED STATES OF AMERICA) 16 November 1989 see claims ---	1-17
	-/--	
<p>¹⁰ Special categories of cited documents:</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"&" document member of the same patent family</p>		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search 12 OCTOBER 1993		Date of Mailing of this International Search Report 15. 10. 93
International Searching Authority EUROPEAN PATENT OFFICE		Signature of Authorized Officer NOOIJ F.J.M.

Form PCT/ISA/210 (second sheet) (January 1985)

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III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
A	<p>PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE USA vol. 87, no. 16, August 1990, WASHINGTON DC, USA pages 6363 - 6367 J. ELLIOTT ET AL. 'Genes for Plasmodium falciparum surface antigens cloned by expression in COS cells.' see abstract see page 6367, left column, line 31 - line 35</p> <p style="text-align: center;">---</p>	1-17
A	<p>PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE USA vol. 88, no. 21, 1 November 1991, WASHINGTON DC, USA pages 9533 - 9537 B. WIZEL ET AL. 'Identification of a continuous and cross-reacting epitope for Plasmodium falciparum transmission-blocking immunity.' see abstract</p> <p style="text-align: center;">---</p>	1-17
P,X	<p>THE JOURNAL OF EXPERIMENTAL MEDICINE vol. 177, no. 2, 1 February 1993, NEW YORK, USA pages 505 - 510 P. DUFFY ET AL. 'Pgs28 belongs to a family of epidermal growth factor-like antigens that are targets of malaria transmission-blocking antibodies.' see the whole document</p> <p style="text-align: center;">-----</p>	1-17

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 93/06464

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 1-4 and 8-11 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

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